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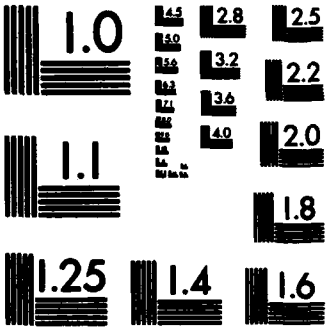

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Studies have begun on the conditioning of identified neurons in the leech Hirudo and on the sea hare Aplysia, and studies have begun on the sea lung Pleurobranchaea on an aspect of the proposed research common to the conditioning of identified neurons system, i.e., those that produce several behaviors by means of the same constituent neurons. In the studies on Hirudo, evidence has been obtained for conditioning in identified neurons that receive inputs from equally identifiable sensory neurons that convey to the central nervous system the conditioned and unconditioned stimuli

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used in training. At least in one circuit, the neuron that produces the reinforcement has been identified. These changes, however,, are short-term, lasting usually no longer than a few seconds to several minutes after training. In further experiments, alredy begun, the neuroeffective substances related to stress that may convert the short-term changes into long-lasting ones will be examined. Similar experiments have been done in Aplysia, but these are presently too few in number (20 as opposed to 40 on Hirudo) from which to make definite statements.

AFOSR Grant 82-0043
Final Report
June, 1983

THE NEURONAL BASIS OF LEARNING

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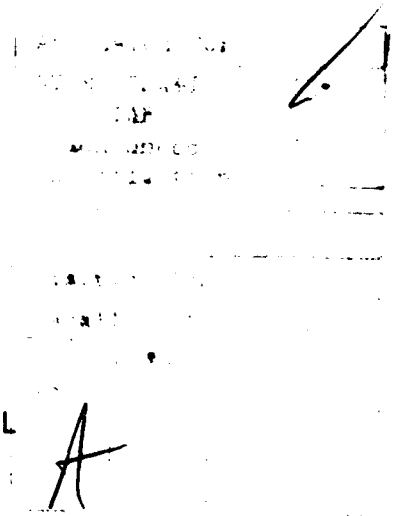

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Final Report

(January 1, 1982-January 9, 1983)

Abstract

During the past year I have established our new laboratory at the Oregon State University Marine Science Center in Newport, begun extensive studies on the conditioning of identified neurons in the leech Hirudo collaboratively with Prof. Kristan at UCSD, begun similar studies on the sea hare Aplysia collaboratively with Prof. Lukowiak at the University of Calgary (both of which efforts are described in the present grant), and begun experiments individually here in Newport on the sea slug Pleurobranchaea on an aspect of the proposed research common to the conditioning of identified neurons and on the analysis of the integrative properties of multifunctional nervous system, i.e., those that produce several behaviors by means of the same constituent neurons. In the studies on Hirudo, we have obtained evidence for conditioning in identified neurons that receive inputs from equally identifiable sensory neurons that convey to the central nervous system the conditioned and unconditioned stimuli used in training. At least in one circuit, we have identified the neuron that produces the reinforcement. These changes, however, are short-term, lasting usually no longer than a few seconds to several minutes after training. In further experiments, already begun, we will look at neuroeffective substances related to stress that may convert the short-term changes into long-lasting ones. Similar experiments have been done in Aplysia, but these are presently too few in number (20 as opposed to over 40 on Hirudo) from which to make definite statements. In Pleurobranchaea, I have been examining the convergence among brain interneurons. The analysis of this convergence will form part of the foundation for conditioning of small nerve networks.

In a related series of experiments, we have been examining (in collaboration with Prof. S.B. Kater of the University of Iowa) the formation of synapses between identified neurons during regeneration in the snail Helisoma. By the use of axonal regeneration, we are forcing cells, which normally do not interconnect, to connect with one another. In line with our stated research goals, we shall examine how these newly formed synapses are affected by conditioned activity.

All of these studies on the various aspect of the cellular basis of associative learning shall continue under contract AFOSR F49620-83-C-0063.

Final Report

During the first year of the present grant the goal has been 1) to establish our laboratory at the Oregon State Marine Science Center in Newport, and 2) to make headway into the central issues of the proposed research.

1. The Development of the Laboratory

During the past year I have completed the construction of one of the two proposed neurophysiological work stations. The second work station is largely completed and is being modified so as to interface with a monitoring and controlling computer. Owing to the results, described below, of studies conducted collaboratively with Prof. William Kristan, at UCSD, and Prof. Ken Lukowiak, at the University of Calgary, reasons for extending the capabilities of the laboratory have arisen. I have begun a long-range program to apply computer technology to the acquisition of physiological data for doing real-time interactive studies on experimental preparations.

2. Progress in the Physiological Studies

The proposed research has two features or experimental goals that are at the center of all the intended behavioral and physiological studies. The first has to do with associational conditioning of small neurocircuits. The second has to do with the experimental and conceptual understanding of multifunctional nervous systems; that is, on how the nervous system produces different behaviors using the same constituent neurons which are themselves multifunctional. Functions such as command and coordination are distributed among many neurons rather than conferred to single neurons. A careful consideration will show that these two features are interrelated, for the elucidation of how associational conditioning in small networks of cells is expressed behaviorally requires an understanding of how the nervous system generates the behavior itself. The one problem is embedded in the other.

Work has progressed on both of these problems. In the conditioning of networks, over forty experiments have been conducted collaboratively with Prof. Kristan, twenty experiments with Prof. Lukowiak, and I have individually begun to look at the convergence of the paracerebral cells (PCC) onto the buccal-cerebral interneurons (BCI) in Pleurobranchaea as a prelude to both the integrative and conditioning aspects of the proposed research.

In the conditioning of identified neurons in the leech, we have used small, reduced preparations composed of several segments of skin, attached sensory-motor nerves, and one segmental ganglion, as illustrated in Fig. 1. In these preparations it is possible to identify motoneurons and interneurons that receive monosynaptic inputs from several sensory cells whose cell bodies are identifiable in the ganglion and whose distal terminals innervate specific areas of the skin.

Our first set of studies involved the use of one intracellular electrode, inserted into a follower neuron, such as the PAG cell shown in Fig. 1, that recorded the conditioned responses. The UCS- and CS-equivalent stimuli were applied to areas of the skin so as to activate the various sensory cells selectively. By adjusting the location of the skin electrodes and the voltages that were applied through them, we were able to activate the P or T (pressure or touch) cells individually or together to provide the stimulus for the CS. The CS usually produced small postsynaptic potentials (psps) in the follower neuron (Fig. 1A). For the UCS, short trains of pulses were applied through the other skin electrode (Fig. 1B and C). The UCS produced large psps in the follower neuron and reinforced changes in the response of the follower neuron to the CS (Fig. 1B and C). The production of the large psps with the UCS required much higher stimulus voltages than those applied with the CS. This suggested that the sensory receptor responsible for conveying the UCS to the central nervous system was one of the nociceptive cells (N) since lower stimulus voltages that activated only the P and T cells did not change the response of the follower cell to the CS. We could determine exactly which sensory cells were activated in both the CS and UCS because their characteristic action potentials could be recorded from the dorsal nerve roots with extracellular suction electrodes (a usual procedure in our experiments, but not shown in Fig. 1 in order to simplify the schematic). Intracellular recordings were also made at some time during the experiments from the sensory cell bodies themselves to verify which of the sensory cells were being activated by the CS and UCS. In subsequent studies we demonstrated directly that the N cell provides the UCS to the PAG neuron (other follower cells have not been tested yet in conjunction with the N cell) by driving the N cell directly by means of depolarizing currents passed through the intracellular electrode.

The rationale behind the technical approach in these studies is first to establish the validity of the conditioning procedure in identified neurons by means of the simpler skin-stimulation preparation. In subsequent experiments we shall use the more difficult and time-consuming method of presenting the CS and UCS through recording/stimulating microelectrodes placed in the input cells of the follower neuron, as described above in the identification of the N cell as the source of the UCS to the PAG. Other such experiments will show whether the P or T cells, or both, act as the CS input.

The conditioning studies on the leech have uncovered evidence only of short-term changes in response to the CS, as illustrated in Fig. 1. This may explain why backward conditioning, in which the UCS briefly precedes the CS, is more effective than forward conditioning in which the CS precedes the UCS. In backward conditioning the affects produced by the closely juxtapositioned UCS can contribute to the CS whereas in forward conditioning the CS appears in one conditioning trial long after the affects of the UCS from the preceding trial have waned. Obviously, the best and, perhaps, only way to compare backward and forward conditioning is in preparations that exhibit long-term associative changes.

The production of such long-term changes is in fact our present goal in the experiments on the leech. We believe that factors related to stress may change the short-term responses into long-term ones. Stress may well be an integral part of all long-term associative learning, and may be a consequence of all strong USCs, whether they are positive or negative ones (see Mpitsos, Collins and McClellan, Science 199, 1978, p. 497, for a discussion of the qualities of CSs and UCSs). In Pleurobranchaea, for example, mild noxious UCSs produce only short-term associative changes, whereas stronger UCSs that greatly affect the performance of the animals produce long-term changes (unpublished observation).

To test this possibility we have begun to use neuroeffective substances such as serotonin that can be applied either to the bathing medium of the experimental preparation or selectively released by stimulating identified neurons that contain the substance. Although we have obtained changes in the size and shape of conditioned psps during the application of serotonin, it is too early in these studies to make definite conclusions. In parallel studies, the media perfusing the preparation will contain hemolymph taken from animals that themselves have been stressed. By such a combined approach of selective application of known neuroeffective substances and the fractionation of blood from stressed animals we hope to obtain some consistent evidence of factors that aid in the production of long-term associative changes.

In the studies on Aplysia, conducted collaboratively with Prof. Lukowiak, the first set of experiments have involved, as in the leech, the use of sensory cells that synapse directly on identified motoneurons, such as gill motoneurons L7, L9, and L10. Here, more than in the leech, there is the complication that the responses produced by the sensory cells on the motoneurons rapidly habituate. Whether this problem can be overcome or used to advantage remains to be seen, and, in defense of using the sensory cells in a few more experiments, it is too early to make definite decisions.

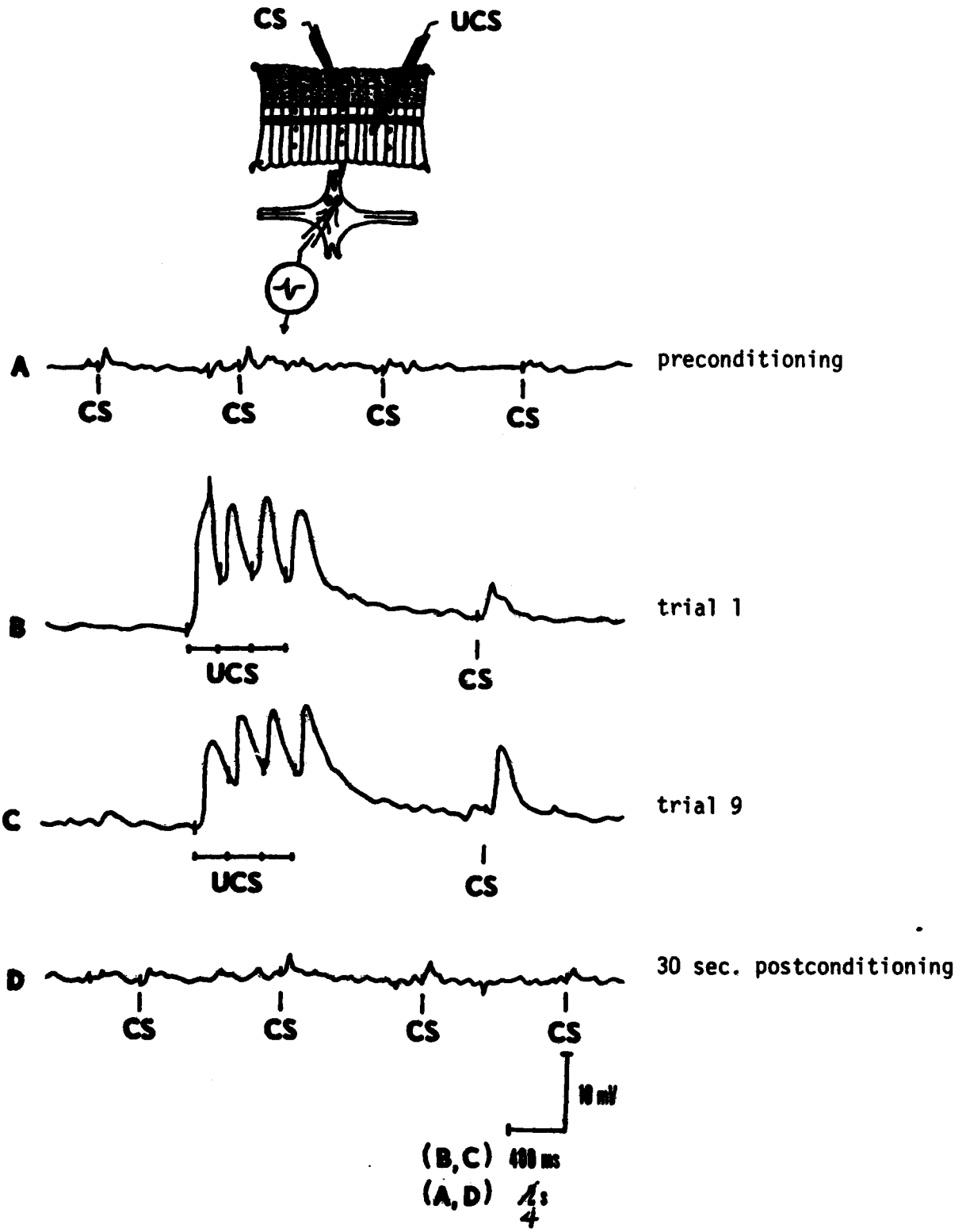
One of the important consequences of the Aplysia work has been the demonstration of the need of automated, computerized data acquisition for on-line interactions with the experimental preparation. The use of such methods at the University of Calgary has shown that changes thought initially to provide evidence of conditioning were later shown to be contrary. In other instances the same methods uncovered the possibility of small conditioned changes that could not have been observed without the aid of automated data acquisition.

In the work on Pleurobranchaea, I have begun to establish the evidence for, and examine the properties of, convergence among the PCC onto the BCI. The property of convergence is a central issue in both the conditioning and the integrative aspects of the proposed research. We already have evidence of excitatory and inhibitory PCC on the BCI, but the evidence is at present indirect as it has been assessed by the responses of the cells that are driven by the BCI. Once these converging neurons have been analyzed, the PCC and the BCI will constitute one set of neurons that will be used for conditioning. Cohan and Mpitsos (1982 in J. Expl. Biol. 102, 25-42; 102, 43-57) discuss the

implications of convergence in neurons systems that produce different behaviors by means of the same sets of neurons.

3. Use of Regeneration-Induced Synaptogenesis in Conditioning Studies

Some of the neurons in the snail Helisoma normally do not synapse with one another. In collaboration with Prof. S.B. Kater's laboratory at the University of Iowa we are obtaining evidence that some of these reidentificable neurons form synapses with one another after their axons are cut. Experimentally, we crush certain nerve trunks in partially dissected animals, and then close the animal with a series of small sutures. Kater's laboratory has shown that neurons which have axons in the crushed nerve trunks sprout new projection and form synapses on other neurons. Two days after the nerve trunks are crushed, we remove the nervous system and examine them for the new connections. Our goal in these studies is to determine whether the formation of the newly formed synapses can be affected by temporally paired activity induced by driving the cells electrically while we are simultaneously recording their physiological responses.



Experiments conducted on the leech Hirudo medicinalis in collaboration with Dr. William Kristan at UCSD.

Top part of the figure illustrates the experimental preparation. A-D illustrate the intracellular responses obtained from identified neurons in a central ganglion. Preconditioning Tests: A. CS stimuli evoke only

Legend to Fig. 1 continued

small, marginally observable depolarizing responses in the target cell (here identified as the pagoda neuron). Conditioning: B-C. When presenting the UCS to the skin (about 1 sec. before the CS test) the response to the CS becomes almost as large as the response to the UCS. Postconditioning: D. This change requires pairing of the two stimuli, and is short-term, lasting only about 1 min. after pairing. Experiments are underway which are aimed at obtaining longer-lasting responses. In the meantime, we have found that the sensory cells that carry the CS are the touch and pressure sensitive neurons (identified in the ganglion) while the cell that carries the UCS is the nociceptive sensory cell (also identified in the ganglion).

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